PRE-IMPLANTATION ABNORMALITIES IN THE MOUSE AND THE OPOSSUM OVUM

BY

GERTRUDE RAFFERTY MACHLIS

B.S., Mundelein College, 1937

M.S., University of Illinois, 1943

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY IN THE GRADUATE SCHOOL OF THE UNIVERSITY OF ILLINOIS,

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PRE-IMPLANTATION ABNORMALITIES IN THE MOUSE AND THE OPOSSUM OVUM

A study has been made of pre-implantation abnormalities arising spontaneously in the ovum of *Didelphis virginiana* and the laboratory mouse and the bearing of such abnormalities on the prenatal death rate in these forms. An analysis of the records of 203 female opossums yielding fertile eggs, embryos, and pouch young places the prenatal death rate at 68%. An average of 16 eggs per female were ovulated during estrus of which 4.4 were unfertilized, and 1.6 began development but did not progress beyond the stage of differentiation of the germ layers. Of a total of 135 abnormal ova recovered, 100 of these eggs have been studied in serial section, and classified as to the stage and type of abnormality. No peak in abnormal development could be detected. The specimens are rather evenly distributed over the stages from early cleavage to the formation of the first somite. In addition to the large number of eggs which died during cleavage or germ layer formation, there are included specimens which deviated in mode of development—one-half normal blastocysts, false morulae, twin vesicles, and eggs in which tumor-like growth has resulted from too rapid cell proliferation. In about one-fourth of the abnormal eggs, the embryonic region is dead or markedly reduced, while the trophoblastic region remains relatively normal. This is discussed in terms of cell lineage in *Didelphis*.

The eggs secured from 118 mice were studied in hanging drop preparations with vital stains. These animals were divided into three groups: those which had just reached breeding age, those of optimum breeding age, and those approaching senility. Most of the mice were examined more than 70 hours post-plug, by which time the eggs could be expected to have reached the uterus and begun to implant. The eggs remaining free in the uterus were then examined and a separation made between the morphologically normal and abnormal. A total of 117 abnormal or dead eggs which had been fertilized and begun development were recovered. In most cases the eggs were aberrent morphologically and in addition could be distinguished from the normal littermates on the basis of their response to vital stains. As a general rule, the dead or dying eggs failed to pick up neutral red or to reduce the oxidized form of methylene blue to the colorless state, while the normal littermates showed the opposite response. In some cases a differential

response was secured within the egg. Studies have indicated that the blastomeres of the mammalian egg are equipotential up to the blastocyst stage; a differential response to vital stains on the part of the blastomeres of the pre-blastocyst stage has been interpreted as indicating a lowered rate of metabolism in certain areas, and the onset of death. Earlier workers have secured a differential response between areas in the early implanted blastocyst. In the 26 pre-implantation blastocysts encountered in this study, which gave a differential response to vital stains within the egg proper, no consistent pattern of response was evidenced; judgment is suspended as to whether these blastocysts are normal.

The greatest variation in breeding behavior as well as the largest number of abnormal zygotes was observed in the youngest group, i.e., females of 2-3.5 months of age. In ten cases the uterine horns were either completely unstimulated, or were stimulated unilaterally so that even where the eggs of the litter appeared potentially viable, successful implantation would not be likely to occur. In six of these females mating was either not accompanied by ovulation or only 1-2 eggs were released. In one young female from which a total of 24 abnormal or unfertilized eggs were secured 123 hours post-plug, four miniature eggs were found, the vitelli of which had an average diameter of approximately one-third that of a normal egg.

In 16 cases, distributed with no apparent relationship to the age of the female, cumulus-surrounded eggs, assumed to represent eggs of the next cycle, were recovered from the Fallopian tubes of the mice more than 70 hours post-plug. In nine of these cases, either fertilized eggs or implants were recovered in the uterus. Here copulation apparently failed to set off the series of changes associated with pregnancy or pseudo-pregnancy, and to inhibit the onset of the next cycle, a condition experimentally produced in the rat by Ball. One case of mating and ovulation during pregnancy was observed, though no eggs were recovered. This has been interpreted as due to a lessened inhibition by the regressing corpora lutea of FSH production by the pituitary as pregnancy neared term.

This study indicates that fertilized eggs in a litter differ in viability so that there is a steady loss of eggs from the beginning of cleavage to the time of implantation. In a few cases an environmental basis for the production of such dead or abnormal eggs may exist, especially where whole litters are abnormal, where the

tract is unstimulated and hence unreceptive to implantation, or where the tract is anatomically abnormal. Where the abnormal specimen is part of a largely normal litter, and where the environment seems adequate to support the growth of the majority, the thesis is advanced that the fault lies not in the environment but in the egg.

VITA

Gertrude Rafferty Machlis was born in Chicago, Illinois, December 22, 1916. After attending public and parochial schools in that city, she enrolled in Mundelein College, Chicago, in September, 1933. The degree of Bachelor of Science cum laude was conferred upon her by that institution in June, 1937. The following September she entered Chicago Teachers College, graduating in February, 1939, with a Chicago Teacher's certificate. From 1939 to 1942 she served as a teacher in the public schools of Chicago, Illinois, and Muskegon, Michigan, and at the French Institute of Notre Dame de Sion in Kansas City, Missouri. During this period she accumulated graduate credit at the University of Chicago.

She entered the Graduate School of the University of Illinois in September, 1942, and received the degree of Master of Science in September, 1943. Since entering graduate school she has served as an assistant in zoology except during the academic year 1943-1944 when she held a fellowship in Zoology. She was married in June, 1946, to Leonard Machlis, now associated with the Botany department of the University of California at Berkeley. She spent the summer of 1946 at the Marine Biological Station at Woods Hole, Massachusetts. She is a member of Phi Sigma, Sigma Delta Epsilon, and an Associate member of Sigma Xi.

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